Experimental design and sample size determination

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Note

- This is a shortened version of a lecture which is part of a web-based course on "Enhancing Humane Science/Improving Animal Research" (organized by Alan Goldberg, Johns Hopkins Center for Alternatives to Animal Testing)
- Few details—mostly concepts.

Experimental design

Basic principles

1. Formulate question/goal in advance
2. Comparison/control
3. Replication
4. Randomization
5. Stratification (aka blocking)
6. Factorial experiments

Example

Question: Does salted drinking water affect blood pressure (BP) in mice?

Experiment:

1. Provide a mouse with water containing 1% NaCl.
2. Wait 14 days.
3. Measure BP.

Comparison/control

Good experiments are comparative.

- Compare BP in mice fed salt water to BP in mice fed plain water.
- Compare BP in strain A mice fed salt water to BP in strain B mice fed salt water.

Ideally, the experimental group is compared to concurrent controls (rather than to historical controls).

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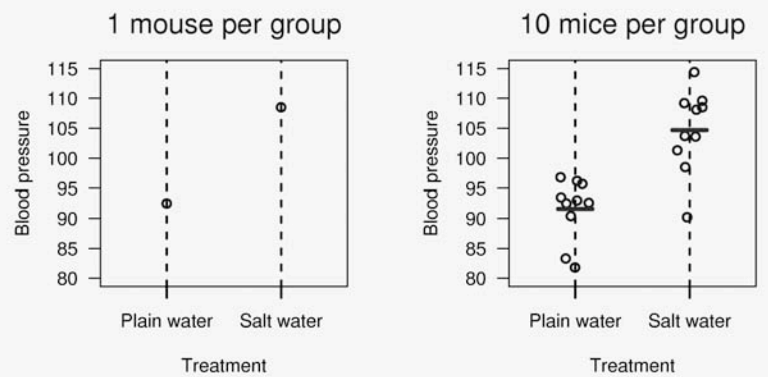
Replication



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Why replicate?

- Reduce the effect of uncontrolled variation (i.e., increase precision).
- Quantify uncertainty.

A related point:

An estimate is of no value without some statement of the uncertainty in the estimate.

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Randomization

Experimental subjects ("units") should be assigned to treatment groups at random.

At random does not mean haphazardly.

One needs to explicitly randomize using
- A computer, or
- Coins, dice or cards.

Why randomize?

- Avoid bias.
  - For example: the first six mice you grab may have intrinsicly higher BP.
- Control the role of chance.
  - Randomization allows the later use of probability theory, and so gives a solid foundation for statistical analysis.

Stratification

- Suppose that some BP measurements will be made in the morning and some in the afternoon.
- If you anticipate a difference between morning and afternoon measurements:
  - Ensure that within each period, there are equal numbers of subjects in each treatment group.
  - Take account of the difference between periods in your analysis.
- This is sometimes called "blocking".

Example

- 20 male mice and 20 female mice.
- Half to be treated; the other half left untreated.
- Can only work with 4 mice per day.

Question: How to assign individuals to treatment groups and to days?

An extremely bad design

Week One: M	Tu	W	Th	F	Week Two: M	Tu	W	Th	F
C	C	C	C	C	T	T	T	T	T
C	C	C	C	C	T	T	T	T	T
C	C	C	C	C	T	T	T	T	T
C	C	C	C	C	T	T	T	T	T

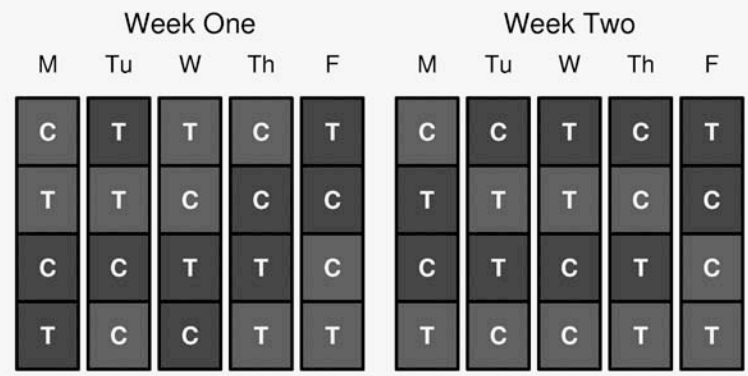
T = treated, C = control, pink = female, blue = male

Randomized

Week One: M	Tu	W	Th	F	Week Two: M	Tu	W	Th	F
T	T	T	T	T	C	T	T	C	T
C	T	T	T	T	C	C	C	T	C
C	C	C	T	T	C	C	T	C	C
T	C	C	C	C	C	T	C	T	T

T = treated, C = control, pink = female, blue = male

A stratified design



T = treated, C = control, pink = female, blue = male

Randomization and stratification

- If you can (and want to), fix a variable.
  - e.g., use only 8 week old male mice from a single strain.
- If you don't fix a variable, stratify it.
  - e.g., use both 8 week and 12 week old male mice, and stratify with respect to age.
- If you can neither fix nor stratify a variable, randomize it.

Factorial experiments

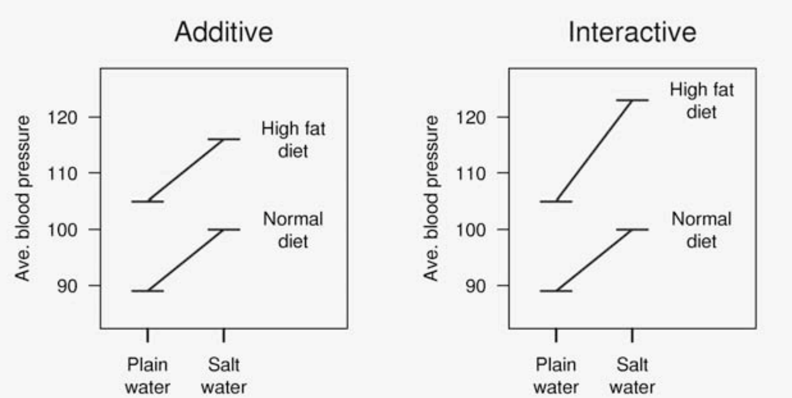
Suppose we are interested in the effect of both salt water and a high-fat diet on blood pressure.

Ideally: look at all 4 treatments in one experiment.

Plain water	×	Normal diet
Salt water		High-fat diet

Why?
- We can learn more.
- More efficient than doing all single-factor experiments.

Interactions



Other points

- Blinding
  - Measurements made by people can be influenced by unconscious biases.
  - Ideally, dissections and measurements should be made without knowledge of the treatment applied.
- Internal controls
  - It can be useful to use the subjects themselves as their own controls (e.g., consider the response after vs. before treatment).
  - Why? Increased precision.

Other points

- Representativeness
  - Are the subjects/tissues you are studying really representative of the population you want to study?
  - Ideally, your study material is a random sample from the population of interest.

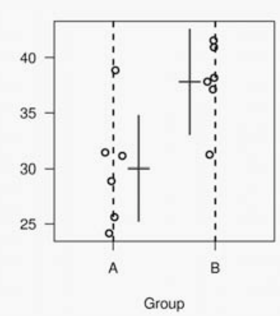
Summary

Characteristics of good experiments:

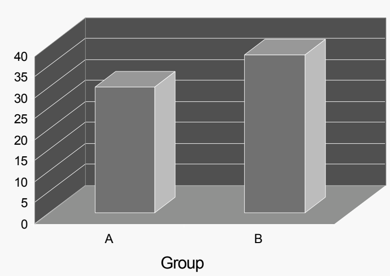
- Unbiased
  - Randomization
  - Blinding
- High precision
  - Uniform material
  - Replication
  - Blocking
- Simple
  - Protect against mistakes
- Wide range of applicability
  - Deliberate variation
  - Factorial designs
- Able to estimate uncertainty
  - Replication
  - Randomization

Data presentation

Good plot



Bad plot



Data presentation

Good table

Treatment	Mean	(SEM)
A	11.2	(0.6)
B	13.4	(0.8)
C	14.7	(0.6)

Bad table

Treatment	Mean	(SEM)
A	11.2965	(0.63)
B	13.49	(0.7913)
C	14.787	(0.6108)

Sample size determination

Fundamental formula

$$n = \frac{\$\text{ available}}{\$\text{ per sample}}$$

Listen to the IACUC

Too few animals → a total waste

Too many animals → a partial waste

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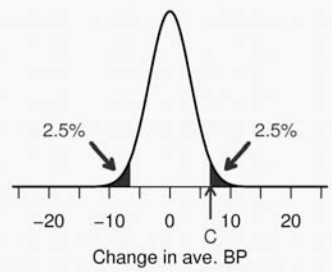
Significance test

- Compare the BP of 6 mice fed salt water to 6 mice fed plain water.
- Δ = true difference in average BP (the treatment effect).
- H_0: $\Delta = 0$ (i.e., no effect)
- Test statistic, D.
- If $|D| > C$, reject H_0.
- C chosen so that the chance you reject H_0, if H_0 is true, is 5%

Distribution of D when $\Delta = 0$



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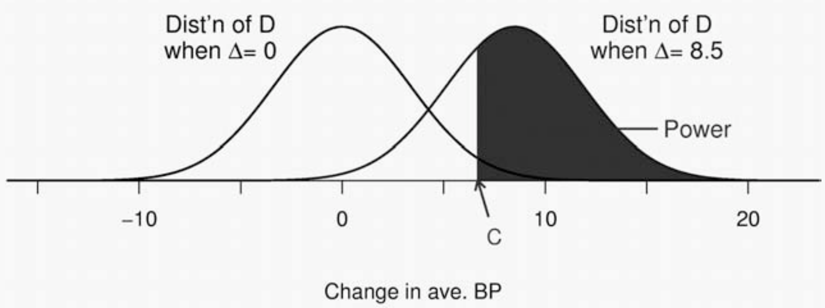
Statistical power

Power = The chance that you reject H_0 when H_0 is false (i.e., you [correctly] conclude that there is a treatment effect when there really is a treatment effect).



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Power depends on…

- The structure of the experiment
- The method for analyzing the data
- The size of the true underlying effect
- The variability in the measurements
- The chosen significance level (α)
- The sample size

Note: We usually try to determine the sample size to give a particular power (often 80%).

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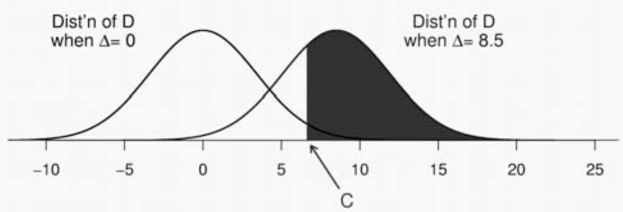
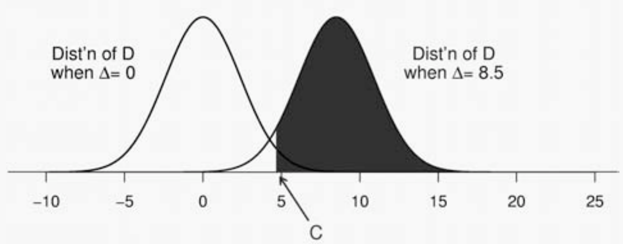
Effect of sample size

6 per group:

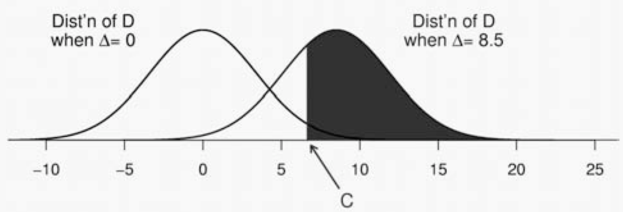


12 per group:

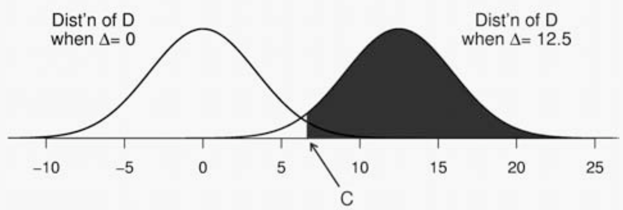


Effect of the effect

Δ = 8.5:



Δ = 12.5:



Various effects

- Desired power ↑ ⇒ sample size ↑
- Stringency of statistical test ↑ ⇒ sample size ↑
- Measurement variability ↑ ⇒ sample size ↑
- Treatment effect ↑ ⇒ sample size ↓

Determining sample size

The things you need to know:

- Structure of the experiment
- Method for analysis
- Chosen significance level, α (usually 5%)
- Desired power (usually 80%)
- Variability in the measurements
  - if necessary, perform a pilot study
- The smallest meaningful effect

A formula

$$n = \left(\sigma \ \ldots\right)^2 \left[\ldots {}_{1-\beta} \right]^2 \times 2$$

Censored

Reducing sample size

- Reduce the number of treatment groups being compared.
- Find a more precise measurement (e.g., average time to effect rather than proportion sick).
- Decrease the variability in the measurements.
  - Make subjects more homogeneous.
  - Use stratification.
  - Control for other variables (e.g., weight).
  - Average multiple measurements on each subject.

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Final conclusions

- Experiments should be designed.
- Good design and good analysis can lead to reduced sample sizes.
- Consult an expert on both the analysis and the design of your experiment.

Resources

- ML Samuels, JA Witmer (2003) *Statistics for the Life Sciences*, 3rd edition. Prentice Hall.
  - An excellent introductory text.
- GW Oehlert (2000) *A First Course in Design and Analysis of Experiments*. WH Freeman & Co.
  - Includes a more advanced treatment of experimental design.
- Course: *Statistics for Laboratory Scientists* (Biostatistics 140.615-616, Johns Hopkins Bloomberg Sch. Pub. Health)
  - Intoductory statistics course, intended for experimental scientists.
  - Greatly expands upon the topics presented here.